Toxicological evaluation of certain food additives

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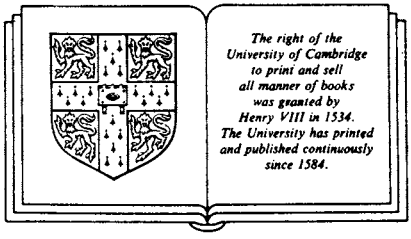

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ENZYME PREPARATIONS

Problems in evaluating the safety of enzymes in food processing were discussed at the fifteenth, eighteenth and twenty-ninth meetings of the Expert Committee, when principles relating to their evaluation were elaborated (Annex 1, references 26, 35, and 70). At its present meeting, the Committee reaffirmed those principles, which have been consolidated in Annex III of "Principles for the Safety Assessment of Food Additives and Contaminants in Food" (Annex 1, reference 76).

For the purpose of toxicological evaluation, the enzyme preparations under present consideration were grouped into the following classes:

- Class III - Enzymes derived from Aspergillus oryzae;
- Class IV - Enzymes derived from Aspergillus niger; and
- Class V - Enzymes derived from Trichoderma reesei, Trichoderma harzianum, Penicillium funiculosum, Aspergillus alliaceus.

The guidelines established by JECFA for these classes of enzymes provide a basis for the toxicological studies required for their evaluation.

At the twenty-ninth meeting the Committee concluded that, when enzyme preparations from either class IV or class V are added directly to food but not subsequently removed, an acceptable daily intake should be established to ensure that levels of the enzyme preparations in food are safe. In order to evaluate the information

received on the estimate of the amount of enzyme preparations used in the toxicological studies and levels of consumption resulting from their use in food, the Committee adopted the concept of enzyme total organic solids (TOS), which is defined as follows: % TOS = 100-(A+W+D), where A = % ash, W = % water, and D = % diluent and carrier (Ad hoc Enzyme Technical Committee, 1981; Pariza & Foster, 1983). This concept overcomes the problem that enzyme preparations of different activities and forms were used in the toxicological studies. It also takes into account that most of the organic solids in this fraction are not the enzyme per se.

In establishing acceptable daily intakes for the enzymes in classes IV and V, the Committee noted that the animal feeding studies were primarily of short-term duration. It, therefore, concluded that it would be appropriate to use a safety factor greater that the usual 100.

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ENZYMES DERIVED FROM ASPERGILLUS ORYZAE

EXPLANATION

Enzymes from this source were considered at the fifteenth meeting of the Committee (Annex 1, reference 26), at which time a decision on the ADI was postponed because of concern that one of the known metabolites of A. oryzae is β-nitropropionic acid, which was suspected of carcinogenic potential. Later, at the eighteenth meeting of the Committee, a lipase derived from this organism was considered (Annex 1, reference 35). It was determined at that time that there was no information to substantiate the concern for the potential carcinogenicity of β-nitropropionic acid, and that analyses of foods have shown that the metabolite is present in very few foods and then only in minute amounts. The present Committee was also informed that A. oryzae varieties are used in certain parts of the world in the preparation of foods.

α-AMYLASE (E.C. 3.2.1)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Acute toxicity

Animal	Route	LD_{50}	Reference
Mouse (Novo Strain)	Oral	> 20 g/kg b.w.	Novo, 1971a

Short-term studies

Rats

Three groups, each containing 5 male and 5 female SPF Wistar rats, were maintained for 3 weeks on diets containing 0, 0,5, or 5% of the enzyme preparation. Only minor differences were observed among the groups in body-weight change and food intake. At termination of the study, haematologic measurements, organ-weights analyses, and gross post mortem examinations showed no compound-related effects (Novo, 1971b).

In another study, two groups, each containing 10 male and 10 female ARS Sprague-Dawley rats, were fed diets containing 5 or 10% of the test enzyme (equivalent to 3.5 or 7 g enzyme/kg/b.w./day) for 90-94 days. A control group of 20 male and 20 female rats was maintained on the diet alone. No signs of toxicity were observed during the test period. Body-weight gain and food consumption were similar among animals in the test and control groups. Differential blood counts were within the normal range at weeks 4 and 8 in all groups. At the end of the study, haematologic parameters, organ-weight analyses, and gross and microscopic pathology showed no compound-related effects (Garvin et al., 1972a).

A similar study was performed with carbohydrases from A. oryzae (α-amylase and amyloglucosidase), prepared under different culture conditions. No compound-related effects were reported (Gavin et al., 1972b).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

Short-term studies on α-amylase from A. oryzae did not reveal any adverse effects. Based upon its lack of toxicity and the fact that A. oryzae varieties are used in the preparation of foods, this enzyme was considered to be acceptable for use in food.

EVALUATION

Level causing no toxicological effects

Rat: 10% in the diet, equivalent to 7 g/kg b.w./day.

Estimate of acceptable daily intake

Acceptable for use in food when used according to good manufacturing procedures.

REFERENCES

Garvin, P.J., Ganote, C.E., Merubia, J., Delahany, E., Bowers, S., Varnado, A., Jordan, L., Hatley, G., DeSmet, C., & Porth, J. (1972a). Unpublished report from Travenol Laboratories, Inc., Morton Grove, IL, USA. Submitted to WHO by Gist-brocades NV, Delft, Holland.

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Novo (1971a). Acute toxicity of fungamyl to mice. Unpublished report from Novo Industri A/S, Bagsvaerd, Denmark. Submitted to WHO by Novo Industri A/S, Bagsvaerd, Denmark.

Novo (1971b). Three week oral toxicity study of fungamyl in rats. Unpublished report BSi/BS from Novo Industri A/S, Bagsvaerd, Denmark. Submitted to WHO by Novo Industri A/S, Bagsvaerd, Denmark.

PROTEASES (E.C. 3.4.21.14; 3.4.23.6)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Acute toxicity

No information available.

Short-term study

Rats

Two groups of 10 male and 10 female ARS Sprague-Dawley rats were fed diets containing 5 or 10% of the test enzyme preparation (equivalent to 3.5 or 7 g enzyme preparation/kg b.w./day) for 90 to 94 days. A control group of 20 male and 20 female rats were maintained on the diet alone. No signs of toxicity were observed during the test period. Body-weight gain and food consumption were similar in animals in the test and control groups. Differential blood counts were within the normal range at weeks 4 and 8 in all groups. At the end of the study serum clinical chemistry parameters, organ weight analyses, and gross and microscopic pathology showed no compound-related effects (Garvin et al, 1972).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

A short-term study in rats on a protease preparation from A. oryzae did not reveal any adverse effects. Based on its lack of toxicity and the fact that A. oryzae varieties are used in the preparation of foods, this enzyme was considered to be acceptable for use in food.

EVALUATION

Level causing no toxicological effect

Rat: 10% in the diet, equivalent to 7 g/kg b.w./day.

Estimate of acceptable daily intake

Acceptable for use in food when used according to good manufacturing procedures.

REFERENCE

Garvin, P.J., Ganote, C.E., Merubia, J., Delahany, E., Bowers, S., Varnado, A., Jordan, L., Hatley, G., DeSmet, C., & Porth, J. (1972). Protease from Aspergillus oryzae. Unpublished report from Travenol Laboratories, Inc., Morton Grove, IL, USA. Submitted to WHO by Gist-brocades NV, Delft, Holland.

ENZYMES DERIVED FROM ASPERGILLUS NIGER

EXPLANATION

A. niger is a contaminant of food and was not considered in the same light as those organisms regarded as normal constituents of food. It is necessary to show that the strains used in enzyme preparations do not produce mycotoxins.

Microbial carbohydrases prepared from some varieties of A. niger were evaluated at the fifteenth meeting of the Committee, at which time a temporary ADI "not limited" was established (Annex 1, reference 26). A toxicological monograph was prepared (Annex 1, reference 27). An adequate 90-day study in rats was requested. Since the previous evaluation, additional data have become available on a number of carbohydrases, which are summarized and discussed in the following monograph. These enzymes were considered by the Committee to encompass the carbohydrases previously considered. The previously published monograph has been expanded and reproduced in its entirety below.

AMYLOGLUCOSIDASES (E.C. 3.2.1.3)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on aflatoxin-related effects

Ducklings

Four groups of 5 ducklings received in their diet 0, 1, 5, or 10% enzyme preparation for 29 days. Growth, feed consumption, survival, behaviour, and mean liver weights were comparable, in all groups. No gross or histopathological lesions of the liver were seen (FDRL, 1963a).

Four groups of 5 ducklings received in their diet 0, 1, 5, or 10% enzyme preparation for 29 days. Growth, feed consumption, survival, behaviour, and development were comparable in all groups. No gross liver lesions were seen at autopsy and mean liver weights of treated animals were similar to those of controls. Histopathology of the livers was normal. No toxic elements were noted (FDRL, 1963b).

Acute toxicity[1]

Species	Route	LD_{50} (mg/kg b.w.)	Reference
Mouse	oral	> 3,200	Hunt & Garvin, 1963
		> 4,000	Hunt & Garvin, 1971
		> 3,200	Willard & Garvin, 1968
		> 4,000	Garvin et al., 1966
Rat	oral	10,000	Gray, 1960
		31,600	Kay & Calendra, 1962
		> 3,200	Willard & Garvin, 1968
		> 4,000	Garvin et al., 1966
		12,500 - 20,000	Kapiszka & Hartnage, 1978
Rabbit	oral	> 4,000	Garvin et al., 1966
Dog	oral	> 4,000	Garvin et al., 1966

[1] These data were obtained with several different commercial enzyme preparations.

Short-term studies

Rats

Three groups of 10 male rats received 0, 0.5, or 5% enzyme preparation in their diets for 30 days. No adverse effects related to treatment were observed regarding growth, appearance, behaviour, survival, food consumption, haematology, organ weights, or gross pathology (Garvin et al., 1966).

Two groups of 10 male and 10 female rats received either 0 or 5% enzyme preparation in their diets daily for 91 days. No differences from controls were observed regarding appearance, behaviour, survival, weight gain, haematology, organ weights, or gross pathology (Garvin & Merubia, 1959).

Two groups of 10 male and 10 female ARS Sprague-Dawley rats were fed diets containing 5 or 10% of the test enzyme preparation (equivalent to 3.5 or 7 g enzyme preparation/kg b.w./day) for 90 to 94 days. A control group of 20 male and 20 female rats were maintained on the diet alone. No signs of toxicity were observed during the test period. Body-weight gain and food consumption were similar between test and control groups. Differential blood counts were within the normal range at weeks 4 and 8 of the study in both test and control animals. At the end of the study serum clinical chemistry parameters, organ weight analyses, and gross and microscopic pathology showed no compound-related effects (Garvin et al, 1972).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

Several short-term feeding studies in rats on amyloglucosidase preparations from A. niger have been performed. One study, in which the preparation was fed at up to 10% of the diet, was

considered to be acceptable by current standards. No compound-related effects were observed in this study or in duckling tests that were performed to investigate potential aflatoxin-related effects.

The evaluations by the Committee of the carbohydrases and the protease from A. niger are summarized at the end of this section.

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β-GLUCANASE (E.C. 3.2.1.6)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies (The TOS of the enzyme preparation used for toxicity studies was 49%).

Special studies on mutagenicity

The enzyme preparation was tested for mutagenic activity using 5 strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and TA1538 both with and without metabolic activation (S-9 fraction). The preparation was not mutagenic or toxic at concentrations up to 40 mg/ml (McConville, 1980).

A cytogenic bone marrow study was performed using adult male Chinese hamsters. Groups of adult male hamsters received up to 5000 mg/kg b.w./day of the enzyme preparation for 5 consecutive days. Treatment did not result in an increased frequency of chromosomal aberrations in bone marrow (McGregor & Willins, 1981).

Acute toxicity

Species	Route	Sex	LD_{50} (ml/kg b.w.)	Reference
Mouse (NMRI)	oral	M & F	30	Novo, 1978a
Rat (Wistar)	oral	-	28.1	Novo, 1978b

Short-term studies

Rats

Three groups, each containing 5 male and 5 female Wistar/Mol SPF rats, were dosed orally by gavage once a day for 14 days with enzyme preparation at dose levels equivalent to 2.5, 5.0, or 10 ml/kg b.w. No clinical changes were observed. Body-weight gains of test and control animals were similar. At termination of the study, measurements of organ weights showed no compound-related effects (Novo, 1978c).

In another study, 4 groups, each containing 15 male and 15 female Wistar/Mol SPF rats, were dosed by gavage once a day for 90 days with enzyme preparation at dose levels equivalent to 0, 2.5, 5.0, or 10 ml/kg b.w. Deaths, primarily in the high-dose group, appeared to be related to injury during dosing. No clinical signs were observed in the other test animals. Male rats in the high-dose group showed decreased weight gain and marked decrease in food intake. Haematology studies showed increased platelet counts and decreased clotting times in the high-dose group at week 6, but this effect was not apparent at week 12. No other effects were reported. Clinical chemistry and urinalysis values at weeks 6 and 12 were within the normal range. At termination of the study, organ weight analysis showed a marked increase in relative weights of the spleen and testes of the males in the high-dose group. Gross and histopathological examination of the principal organs and tissues showed no compound-related effects (Perry et al., 1979).

Dogs

Three groups, each containing one male and one female beagle dog, received single doses of 5, 10, or 15 ml/kg b.w. of the enzyme preparation over a 4-day period. Following a 7-day observation period the dogs were sacrificed and subjected to macroscopic post-mortem examination. No compound-related effects were observed, with the exception of vomiting during the first 4 days of the study. In another study, dogs were administered consecutive doses of 15 ml/kg b.w./day for 9 days, and 10 ml/kg b.w./day for 5 days. No deaths occurred during the course of the study. The only clinical sign noted was

excessive salivation and emesis shortly after dosing. Body weights, electrocardiograms, haematological parameters, blood serum chemistry, organ weights, gross pathology, and histopathology showed no compound-related effects (Osborne et al., 1978).

In another study, three groups, each containing 3 male and 3 female beagle dogs, were dosed with the enzyme preparation by gavage once a day, seven days a week, for 13 weeks, at dose levels equivalent to 2, 5, or 8 ml/kg b.w./day. Two dogs in the high-dose group died during the course of the study, which the authors concluded was due to respiratory distress as a result of foreign material in the lungs. Vomiting was reported after dosing in the high-dose group. Haematological parameters at weeks 6 and 12 were within normal limits, with the exception of a significant increase in WBC count, specifically in the group mean neutrophil counts, in the high-dose group. Clinical chemistry values were within the normal range at weeks 8 and 12, with the exception of slight increases in blood glucose and cholesterol in the high-dose group. Urinalysis showed no compound-related effects. At termination of the study, organ-weight analyses and gross and histopathological examination of the principal organs and tissues showed no compound-related effects (Greenough et al., 1980).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

This enzyme preparation was not genotoxic in microbial or in mammalian test systems. Short-term studies in rats and dogs resulted in no observed compound-related effects at levels up to 5 ml/kg b.w./day of enzyme preparation.

The evaluations by the Committee of the carbohydrases and the protease from A. niger are summarized at the end of this section.

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Perry, C.J., Everett, D.J., Cowie, J.R., Maule, W.J. & Spencer, A. (1979). β-glucanase toxicity study in rats (oral administration by gavage for 90 days). Unpublished report No. 1310 from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Novo Industri A/S, Bagsvaerd, Denmark.

HEMI-CELLULASE

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on mutagenicity

The enzyme preparation was tested for mutagenic activity using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 both with and without metabolic activation (S-9 fraction). The test substance was not mutagenic or toxic at concentrations up to 5 mg/plate (Clausen & Kaufman, 1983).

In an in vitro cytogenetic test using CHO-K1 cells, both with and without metabolic activation (S-9 fraction), the enzyme preparation at test levels up to 2.5 mg (dry wt)/ml did not induce chromosomal aberrations (Skovbro, 1984).

Acute toxicity

No information available.

Short-term studies

Rats

Four groups, each containing 5 male and 5 female Wistar MOL/W rats, were dosed by gavage once a day for 90 days with the enzyme preparation at doses equivalent to 0, 100, 333, or 1000 mg/kg b.w./day. No significant clinical changes were observed. Body-weight gain and food intake were similar among test and control animals. Haematologic and clinical chemistry measurements at termination of the study were within normal ranges. Post-mortem examinations, measurements of organ weights, and histopathology showed no compound-related effects. Slight increases in kidney and adrenal weights in the mid-dose group were not associated with histopathological effects, and did not show a dose response (Kallesen, 1982).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

This enzyme preparation was not genotoxic in microbial or in mammalian test systems. In a limited 90-day study in rats, no effects were observed at the highest dose administered (1 g/kg b.w./day). This enzyme preparation contained high levels of pectinase. The pectinase enzyme preparation summarized below may be identical to this hemicellulase preparation, which provides added assurance of the safety of this preparation.

The evaluations by the Committee of the carbohydrases and the protease from A. niger are summarized at the end of this section.

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PECTINASE (E.C. 3.1.1.11; 3.2.1.15; 4.2.2.10)

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies (The TOS of the commercial preparation is approximately 5%).

Acute toxicity

Species	Route	LD_{50} (ml/kg b.w.)	Reference
Rat	oral	18.8-22.1	Porter & Hartnagel, 1979

Short-term studies

Rats

Two groups of 10 male and 10 female ARS Sprague-Dawley rats were fed diets containing 5 or 10% of the test enzyme preparation (equivalent to 3.5 or 7 g of the enzyme preparation/kg b.w./day), for 90 to 94 days. A control group of 20 male and 20 female rats was maintained on the diet alone. No signs of toxicity were observed during the test period. Body-weight gain and food consumption were similar among test and control groups. Differential blood counts at weeks 4 and 8 of the study were within the normal range in test and control animals. At the end of the study serum clinical chemistry analyses, organ weight analyses, and gross and microscopic pathology showed no compound-related effects (Garvin et al., 1972).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

In a short-term study in rats, no adverse effects were observed at dietary levels of the enzyme preparation up to the equivalent of 7 mg/kg b.w./day. This enzyme preparation may be identical to the hemi-cellulase preparation summarized above. The hemi-cellulase enzyme preparation summarized above also contained high levels of pectinase, which provides added assurance of the safety of this preparation.

REFERENCES

Garvin, P.J., Ganote, C.E., Merubia, J., Delahany, E., Bowers, S., Varnado, A., Jordan, L., Hatley, G., DeSmet, C., & Porth, J. (1972). Carbohydrase from Aspergillus niger (pectinase, cellulase and lactase). Unpublished report from Travenol Laboratories, Inc., Morton Grove, IL, USA. Submitted to WHO by Gist-brocades NV, Delft, Holland.

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PROTEASE

No information available.

GENERAL COMMENTS ON ENZYMES FROM A. NIGER

Aspergillus niger is a contaminant of food. Although there may be posible strain differences in A. niger, and different cultural conditions might be used to prepare the various enzymes, the available toxicity data, which consist primarily of short-term feeding studies in rats and some studies in dogs, show that all the enzyme preparations tested were of a very low order of toxicity. The enzyme preparations tested were non-mutagenic in bacterial and mammalian cell systems. Studies on some strains of A. niger used to prepare carbohydrases showed no aflatoxin or related substance production. These studies provide the basis for evaluating the safety of enzyme preparations derived from A. niger. It was also noted that the enzyme preparations tested exhibit a number of enzyme activities, in addition to the major enzyme activity. Thus, there may be considerable overlap of the enzyme activities of the different enzyme preparations so that safety data from each preparation provides additional assurance of safety for the whole group of enzymes.

Since the enzyme preparations tested were of different activities and forms, and most of the organic materials in the preparations are not the enzyme per se, the numerical ADI is expressed in terms of total organic solids (TOS) (see introduction to enzyme preparations section).

EVALUATION

Level causing no toxicological effect

All enzyme preparations tested showed no-observed-effect levels greater than 100 mg TOS/kg b.w./day in 90-day studies in rats.

Estimate of acceptable daily intake

0-1 mg TOS/kg b.w. for each of the enzyme preparations.

BETA-GLUCANASE FROM TRICHODERMA HARZIANUM

EXPLANATION

This enzyme preparation has not been evaluated previously by the Joint FAO/WHO Expert Committee on Food Additives. The preparation used in the toxicological studies were obtained by spray drying the enzyme preparation; the preparation contained 80 - 95% TOS.

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on mutagenicity

The enzyme preparation was tested for mutagenic activity using Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and Escherichia coli WP 2uvra pmK 101 (CM891), both with and without metabolic activation (S-9 fraction). No dose-related increases in revertants were obtained at test levels up to 10 mg/ml of the incubation mixture (Pedersen, 1984).

A cytogenic bone marrow study was performed using adult male Chinese hamsters. Groups of adult male hamsters received up to 5000 mg/kg/day of the enzyme preparation for 5 consecutive days. Treatment did not result in an increased frequency of chromosomal aberrations in bone marrow (McGregor & Holmstrom, 1981).

The enzyme preparation was not mutagenic in mouse lymphoma L5178Y cells at concentrations up to 3.5 mg/ml, with or without metabolic activation (McGregor & Riach, 1984).

Special study on reproduction and teratogenicity

Rats

Groups of 8 pregnant Sprague-Dawley CD rats were used to establish the maternal embryonic maximum tolerated dose. The test animals were dosed daily from day 6 through day 16 of gestation and killed on day 20 of gestation; the number of corpora lutea graviditatis in each ovary and the number of and location of all implantations in the uterus were recorded. The maternal embryonic tolerated dose was established at 5 g/k.g. b.w. For the teratology study, four groups each containing 24 pregnant Sprague-Dawley rats, were dosed daily from day 6 through day 16 (day 17 for controls) with 0, 1, 3, or 8 g/kg b.w. of the enzyme preparation. The rats were killed on day 20 of gestation. Maternal weight gain was reduced at all dose levels of the test compound, and this was accompanied by reduced food consumption in the high-dose group. There were slight reductions in mean litter fetal weight and in mean litter placental weight, which were dose-related. There were no significant dose-related trends in pregnancy data (number of corpora lutea, implantations, resorptions, or live fetuses) or in skeletal abnormalities. However, both the 3 and 8 g/kg b.w. groups showed slight increases in the incidence of hydronephrosis, and the incidence of hydroureter was marginally increased in a dose-related manner. The incidences of these effects, although not significantly different from those in concurrent controls, exceeded the usual background in historical controls (Hazelden & Maddock, 1982).

Acute toxicity

Species	Route	LD_{50} (g/kg b.w.)	Reference
Mouse (Novo)	oral	> 20	Novo, 1975
Rat (Mollegard)	oral	> 10	Novo, 1982

Short-term studies

Rats

Four groups of 15 male and 15 female Charles River CD rats, 4 weeks of age, were maintained for 13 weeks on diet containing 0, 500, 1,500, or 5,000 mg/kg b.w./day of the enzyme preparation. No compound-related deaths were reported. Food intake and body-weight gain were similar in test and control groups, with a tendancy for increased weight gain in females in the high-dose group during the last weeks of the test. Opthalmoscopic examinations at weeks 0 and 12 showed no abnormalities. Haematologic, clinical chemistry, and standard urinalysis values were within normal ranges. However, urinary alkaline phosphatase levels were increased in male rats in the two high-dose level groups at week 12 of the study. Liver cytochrome P-450 measurements showed no evidence of enzyme induction. At termination of the study, no compound-related changes were observed after organ weight analysis and gross and microscopic examination of the principal organs and tissues, with the exception of a slight increase in the incidence of inflammatory reactions in the kidney cortex in the high-dose groups (Warwick et al., 1976).

Dogs

Four groups of 3 male and 3 female dogs were dosed with the enzyme preparation by gavage once a day 7 days a week for 13 weeks at dose levels equivalent to 0, 300, 1000, or 3000 mg/kg b.w./day. Vomiting was reported in the high-dose group. No other clinical signs were observed. Decreased weight gain was observed in female dogs in the two high-dose groups. Although food consumption was also decreased in these groups, the reduced body weight in the high-dose group was greater than expected from the decreased food intake. Haematologic, clinical chemistry, and urinalysis values at weeks 6 and 12 showed no compound-related effects, with the exception of increased urinary alkaline phosphatase at week 12 of the study. At termination, gross necropsy, organ weight analysis, and microscopic examination of the principal organs and tissues showed no treatment-related effects. Liver cytochrome P-450 measurements showed no evidence of enzyme induction (Edwards et al., 1976).

Long-term studies

No information available.

Observations in man

No information available.

COMMENTS

The beta-glucanase preparation was not mutagenic in bacterial or in mammalian systems. The preparation caused no adverse effects in a reproduction study in rats at levels up to 5%, and it was not teratogenic in a rat study at doses up to 1 g/kg b.w./day. Short-term studies showed no adverse effects at 3 g/kg b.w./day in dogs or at 2 g/kg b.w./day in rats. Based on the available information, the Committee established a temporary ADI for this enzyme preparation.

Because this enzyme is derived from a microorganism that is neither a normal constituent of food nor a common contaminant in food, in accordance with Annex III of "Principles for the Safety Assessment of Food Additives and Contaminants in Food" (Annex 1, reference 76), this preparation requires the submission of results of a long-term study in a rodent species as well as specifications to show that the organism does not produce antibiotics and is non-pathogenic to man.

EVALUATION

Level causing no toxicological effect

Rat: 1000 mg/kg b.w./day (teratogenicity study).

Estimate of temporary acceptable daily intake

0-0.5 mg TOS/kg b.w.

Further work or information

Required (by 1992)

1. Long-term feeding study in a rodent species.

2. Additional information to show that this organism does not produce antibiotics and is non-pathogenic to man.

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CELLULASE FROM TRICHODERMA REESEI

EXPLANATION

This substance has not been evaluated previously by the Joint FAO/WHO Expert Committee on Food Additives. Cellulase is produced extracellularly by T. reesei (QM6a), a mutant of T. viride. The enzyme preparation is characterized by two activities, exo-cellobiohydrolase (E.C. 3.2.1.1) and 1,4-endoglucanase (E.C. 3.2.1.4).

Tests have been performed to show that the strain of T. reesei used for the production of this enzyme preparation does not produce any antibiotics, and it can be regarded as non-pathogenic.

The enzyme preparation used in the toxicological studies was a spray dried product derived from a cruder preparation than the commercial product. The TOS of the tested product was 31%.

The available safety data have been summarized by Hjortkjer et al., 1986.

BIOLOGICAL DATA

Biochemical aspects

No information available.

Toxicological studies

Special studies on mutagenicity

The mutagenicity of the enzyme preparation was tested using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, both with and without metabolic activation by S-9 fraction from rat